528 *Inorg. Chem.* **2001,** *40,* 528−532

# Pressure Dependence of Peroxynitrite Reactions. Support for a Radical Mechanism

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*Received November 24, 1998*

Activation volumes ($\Delta V^{\ddagger}$) have been determined for several reactions of peroxynitrite using the stopped-flow technique. Spontaneous decomposition of ONOOH to $NO_3^-$ in 0.15 M phosphate, pH 4.5, gave $\Delta V^{\ddagger} = 6.0 \pm 0.7$ and $14 \pm 1.0$ $cm^3\ mol^{-1}$ in the presence of 53 $\mu$M and 5 mM nitrite ion, respectively. One-electron oxidations of $Mo(CN)_8^{4-}$ and $Fe(CN)_6^{4-}$, which are first order in peroxynitrite and zero order in metal complex, gave $\Delta V^{\ddagger} = 10 \pm 1$ and $11 \pm 1$ $cm^3\ mol^{-1}$, respectively, at pH 7.2. The limiting yields of oxidized metal complex were found to decrease from 61 to 30% of the initially added peroxynitrite for $Mo(CN)_8^{3-}$ and from 78 to 47% for $Fe(CN)_6^{3-}$ when the pressure was increased from 0.1 to 140 MPa. The bimolecular reaction between $CO_2$ and $ONOO^-$ was determined by monitoring the oxidation of $Fe(CN)_6^{4-}$ by peroxynitrite in bicarbonate-containing 0.15 M phosphate, pH 7.2, for which $\Delta V^{\ddagger} = -22 \pm 4$ $cm^3\ mol^{-1}$. The $Fe(CN)_6^{3-}$ yield decreased by ~20% upon increasing the pressure from atmospheric to 80 MPa. Oxidation of $Ni(cyclam)^{2+}$ by peroxynitrite, which is first order in each reactant, was characterized by $\Delta V^{\ddagger} = -7.1 \pm 2$ $cm^3\ mol^{-1}$, and the thermal activation parameters $\Delta H^{\ddagger} = 4.2 \pm 0.1$ kcal $mol^{-1}$ and $\Delta S^{\ddagger} = -24 \pm 1$ cal $mol^{-1}\ K^{-1}$ in 0.15 M phosphate, pH 7.2. These results are discussed within the context of the radical cage hypothesis for peroxynitrite reactivity.

## Introduction

Recognition that peroxynitrite may be both a major pathogenic agent in human diseases associated with oxidative stress[1] and a natural microbicidal agent[2−5] has triggered a renaissance of interest in its chemical properties.[6] This powerful oxidant, which is thought to be formed by coupling of $NO^{\bullet}$ and $O_2^{\bullet-}$ in tissues or cells that simultaneously generate these radicals, reacts by a relatively complex mechanism whose fundamental nature is being actively debated.[6]

The peroxynitrite anion ($ONOO^-$) is relatively unreactive, but upon protonation[1] or addition of Lewis acids[7−11] (including $CO_2$ and certain metal ion complexes) it oxidizes susceptible molecules by one- and/or two-electron processes[12] or, in the absence of reacting partners, it rapidly decomposes to form $NO_3^-$ as the stable product in acidic media.[1,13,14] Kinetic studies have shown that most one-electron oxidations occur by an "indirect" pathway, which is first order in peroxynitrite and zero order in the oxidizable species, indicating that rate-limiting unimolecular activation precedes the oxidation−reduction step.[13,15−20] In contrast, two-electron oxidations appear to occur by a "direct" pathway that is first order in both peroxynitrite and reductant.[12,16,21−24] Maximal yields of oxidized products obtained by the indirect pathway are always significantly less than calculated for stoichiometric conversion by the oxidant. This property indicates the existence of at least two intermediates along the reaction pathway, only one of which undergoes bimolecular reactions, i.e., Scheme 1, where S is a one-electron reductant.

There are two prevailing opinions concerning the nature of the reaction intermediates.[6,12,25] One is that "X" and "Y" are conformational isomers whose reactivities differ from each other, and the other is that "X" and "Y" are caged radical pairs and free radicals formed from homolytic cleavage of the O−O bond, i.e., Scheme 2. One focal point in the debate has been the volume of activation ($\Delta V^{\ddagger}$) measured for the decomposition of ONOOH. This was originally measured to be $\Delta V^{\ddagger} \simeq +1.7$ $cm^3$

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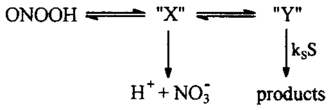
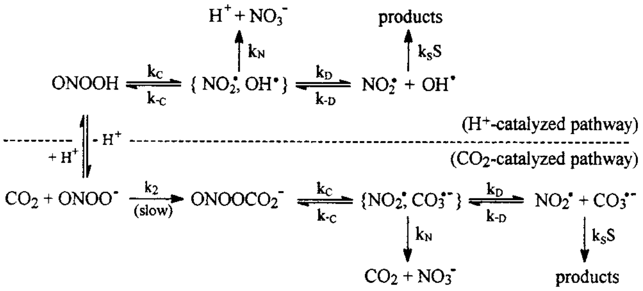
**Scheme 1**



**Scheme 2**



mol$^{-1}$ using a stopped-flow pH-jump technique at pH values ranging from 5.6 to 6.2 and temperatures of 15−23 °C. It was argued that this value is too small to accommodate a mechanism that involves O−O bond homolysis to form a radical pair, but is consistent with an internal rearrangement mechanism.[26] However, pressure-dependent pulse radiolysis experiments, made in 0.15 M formate or low phosphate (4 mM) buffers, have given a self-consistent value for ONOOH decomposition of $\Delta V^{\ddagger} \simeq +10$ cm$^3$ mol$^{-1}$ over the pH range 4.0−7.2.[24,27] As discussed by these researchers,[27] this value is consistent with a radical mechanism. They also demonstrated that $\Delta V^{\ddagger}$ was phosphate-dependent in the pH range 5.6−6.2 and dropped to 1−2 cm$^3$ mol$^{-1}$ when the phosphate concentration was raised to 0.10 M;[27] although the phosphate dependence accounted for the apparent discrepancy in the two studies, the molecular basis for this effect is obscure.

In this paper, we report results from our extensive studies of the pressure dependence of ONOOH reactions measured by stopped-flow spectrophotometry. These data support the radical mechanism, provide evidence for discrete $ONOOCO_2^-$ adduct formation in the reaction between $ONOO^-$ and $CO_2$, and, more generally, are diagnostic of "direct" vs "indirect" reactions of peroxynitrite.

## Experimental Section

**Materials.** All chemicals were reagent grade and were used as received from commercial sources unless otherwise noted; water was purified using a Milli-Q system. Stock solutions of peroxynitrite were prepared from acidified hydrogen peroxide and sodium nitrite using a tandem mixing apparatus,[28] and then stored at −80 °C. Sodium nitrite was present in a 10% excess to minimize contamination by unreacted $H_2O_2$. The stock solution contained ~140 mM peroxynitrite, determined spectrophotometrically ($\epsilon_{302} = 1670$ M$^{-1}$cm$^{-1}$),[29] and 21 mM residual nitrite, determined using the Greiss assay.[30] The metal-complex salts, $K_4Mo(CN)_8$ and $Ni(cyclam)(ClO_4)_2$, were prepared using procedures described in the literature.[31,32]

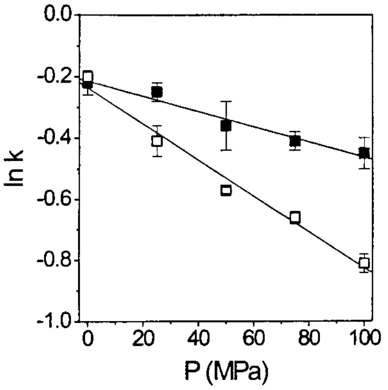


**Figure 1.** Pressure dependence of the first-order rate constant for decomposition of 0.35 mM ONOOH at 19.5 °C in 0.15 M phosphate buffer, pH 4.5, containing 53 $\mu$M (filled squares) and 5.0 mM $NO_2^-$ (open squares). Error bars are equal to twice the standard deviation of four measurements at each pressure; individual rate constants are listed in Table S1. The lines are linear least-squares fit to the data points.

**Kinetic Measurements.** Rate measurements at ambient pressure were made using a Hi-Tech SF-40 stopped-flow spectrophotometer; high-pressure measurements were made using a Hi-Tech HPS-2000 syringe drive unit contained within a high-pressure vessel, as previously described.[33] Both instruments were interfaced to a Hi-Tech SF-40C spectrophotometer control unit. One drive syringe contained 0.3 M phosphate buffer plus desired reductants and/or bicarbonate ion; the other contained the peroxynitrite stock solution that had been appropriately diluted with 10 mM NaOH. In cases where formation of the $ONOOCO_2^-$ adduct was to be avoided, the buffer was sparged with $N_2$ immediately before reaction to remove any accumulated $CO_2$. The final pH was determined both by simulating the mixing conditions outside the stopped-flow apparatus and by measuring the pH of the product solution obtained from the waste syringe. These measurements gave values that were within 0.1 pH unit agreement, and measurements indicated that the buffered reactant solution increased by only 0.1−0.2 pH unit upon mixing with alkaline peroxynitrite. For the high-pressure experiments, the reaction temperature was monitored with a platinum thermistor which was located inside the vessel. Pressures were performed in random order for cases where data for multiple pressures were obtained with a single filling of the syringes. The interval between the preparation of the reactant solutions and the collection of the last data point was always less than 40 min, during which time decomposition of peroxynitrite was <5%. Kinetic data, collected for a minimum of 3−4 half-lives, followed a single-exponential decay profile. Peroxynitrite decomposition in the absence of oxidizable metal complexes was monitored at 280 nm; reactions with $Mo(CN)_8^{4-}$, $Fe(CN)_6^{4-}$, and $Ni(cyclam)^{2+}$ were monitored at 388 nm ($\epsilon_{388} = 1200$ M$^{-1}$cm$^{-1}$),[34] 420 nm ($\epsilon_{420} = 1000$ M$^{-1}$cm$^{-1}$),[35] and 360 nm ($\epsilon_{360} = 5100$ M$^{-1}$cm$^{-1}$),[36] respectively, corresponding to the absorption maxima of the oxidized products. Product yields, calculated from the measured total absorbance changes at these wavelengths, are expressed as the amount of oxidized complex per total amount added peroxynitrite.

## Results

**Decomposition of ONOOH.** At pH 4.5, where virtually all of the peroxynitrite is protonated (p$K_a$ = 6.8), the first-order rate constant for ONOOH decomposition decreased with increasing pressure; typical results are plotted in Figure 1. Activation volumes calculated from the magnitude of the change are $\Delta V^{\ddagger} = 6.0 \pm 0.7$ cm$^3$ mol$^{-1}$ for solutions containing no added $NO_2^-$ (residual $[NO_2^-] = 53$ $\mu$M), and $\Delta V^{\ddagger} = 14 \pm 1$

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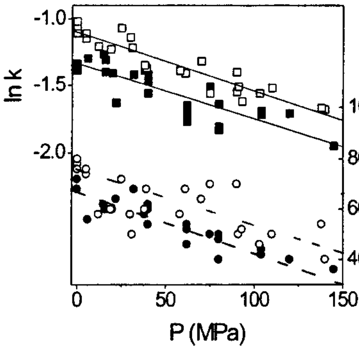

**Figure 2.** Pressure dependence of the first-order rate constant for the reaction in 0.15 M phosphate, pH 7.2, between 0.5 mM ONOOH and 5.0 mM $Mo(CN)_8^{4-}$ (filled squares) at 21 °C or 5.0 mM $Fe(CN)_6^{4-}$ at 25 °C (open squares) and percent yields of $Mo(CN)_8^{3-}$ (filled circles) and $Fe(CN)_6^{3-}$ (open circles). Individual data points are listed in Table S2. The lines are the results of linear least-squares analyses.

$cm^3\ mol^{-1}$ for solutions containing 5 mM $NO_2^-$. The rate constant, measured at atmospheric pressure, was identical to previously determined values ($k = 0.8\ s^{-1}$ at 19.5 °C) and was invariant within ±10% to $NO_2^-$ concentrations measured over the range 53 $\mu$M−10 mM. Previously determined thermal activation parameters for peroxynitrite decomposition at pH 4 are $\Delta H^{\ddagger} = 21$ kcal $mol^{-1}$ and $\Delta S^{\ddagger} = -13$ cal $mol^{-1}\ K^{-1}$.[37]

**Oxidation of Cyanide Complexes of Mo and Fe.** One-electron oxidations of $Mo(CN)_8^{4-}$ and $Fe(CN)_6^{4-}$ are first order in ONOOH and zero order in metal complexes; the observed rate constants are identical to those for ONOOH self-decomposition.[13,16] These properties are diagnostic of the indirect pathway. The maximum yields of the oxidized metal complexes at atmospheric pressure are slightly less than 80% (Figure 2),[16] which correspond well to recent measurements of maximal yields for other "indirect" reactions.[38,39] At pH 7.2, the observed first-order rate constant decreased with pressure over the range 0.1−140 MPa (Figure 2), corresponding to $\Delta V^{\ddagger} = 10 \pm 1\ cm^3\ mol^{-1}$ for $Mo(CN)_8^{4-}$ and $\Delta V^{\ddagger} = 11 \pm 1\ cm^3\ mol^{-1}$ for $Fe(CN)_6^{4-}$. Over the same pressure range, the yield of oxidized metal complexes also decreased proportionately from 61 to 30% for $Mo(CN)_8^{3-}$ and from 78 to 47% for $Fe(CN)_6^{3-}$ (Figure 2). The pressure dependence of $Fe(CN)_6^{4-}$ oxidation was also studied at pH 4.5, up to 125 MPa, with very similar results, i.e., $\Delta V^{\ddagger} = 12 \pm 1\ cm^3\ mol^{-1}$, with the yield decreasing as the pressure increased from 68 to 43% (Figure S1).

The oxidation of $Fe(CN)_6^{4-}$ was also studied at pH 7.2 in phosphate-buffered solutions containing 6 mM bicarbonate ion. Under the prevailing conditions, the $CO_2$ concentration (0.34 mM) exceeded that of peroxynitrite by ~10%, so the reaction proceeded almost entirely via rate-limiting formation of $ONOOCO_2^-$.[8] In this case, the apparent first-order rate constant increased dramatically with increasing pressure up to 82 MPa, for which $\Delta V^{\ddagger} = -22 \pm 4\ cm^3\ mol^{-1}$ (Figure 3). However, the $Fe(CN)_6^{3-}$ yields decreased from ~70 to ~50% over the same pressure range (Figure S2).

**Oxidation of $Ni(cyclam)^{2+}$.** A previously reported study revealed that the rate of oxidation of $Ni(cyclam)^{2+}$ by peroxynitrite was first order in both reagents, indicating a direct bimolecular reaction.[16] From the temperature dependence of the rate constant, determined over the range 5−45 °C, we calculated the thermal activation parameters of $\Delta H^{\ddagger} = 4.2 \pm 0.1$ kcal $mol^{-1}$ and $\Delta S^{\ddagger} = -24 \pm 1$ cal $mol^{-1}\ K^{-1}$ (Figure S3). The rate constant also increased with pressure (Figure 3), for which $\Delta V^{\ddagger} = -7.1 \pm 2\ cm^3\ mol^{-1}$ (Figure 3). The yield of oxidized product was independent of pressure, within experimental uncertainty, and agreed with the value reported earlier for the reaction at atmospheric pressure, i.e., 70−90%.[16]

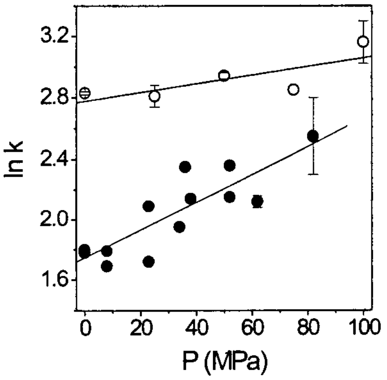

**Figure 3.** Pressure dependence of the pseudo-first-order rate constant for the reaction of 70 $\mu$M ONOOH with 1.0 mM $Ni(cyclam)^{2+}$ at 20 °C (open circles) and the first-order rate constant for reaction of $ONOOCO_2^-$ (0.3 mM peroxynitrite, 6 mM total carbonate) with 5.0 mM $Fe(CN)_6^{4-}$ at 25 °C (filled circles). Both reactions were done in 0.15 M phosphate, pH 7.2. Error bars equal to twice the standard deviation are given for pressures where more than three determinations were made. Individual data points are listed in Tables S3 ($ONOOCO_2^-$) and S4 ($Ni(cyclam)^{2+}$). The lines are the results of linear least squares analyses.

## Discussion

**Radical Nature of the Reactions.** Reactions of ONOOH were initially interpreted in terms of free radical mechanisms;[20,40] however, involvement of the $OH^•$ radical was subsequently questioned on thermodynamic grounds, which led to the development of alternative reaction models, which were based upon differing reactivities of the cis and trans isomeric forms.[12,25] Although these reaction models have been enthusiastically received in some circles, recent studies have clearly demonstrated the radical nature of reactions involving both ONOOH and $ONOOCO_2^-$. Specifically, Merenyi and Lind[41] and we[42] have shown that the highly complex nature of ONOOH decomposition in neutral solutions can be quantitatively simulated by a radical model, the unique feature of which is that *the rate constants for all elementary steps have been independently determined.* This demonstration resolves the debate over whether $OH^•$ formation is thermodynamically feasible;[25,43,44] moreover, it establishes that the radical reaction *will* take place under the prevailing experimental conditions. The radical mechanism has been criticized on the basis that the ONOOH decomposition rate constant failed to decrease upon increasing the medium viscosity,[45] an effect often observed because increasing the viscosity retards primarily escape of the radicals from their solvent cage (the $k_D$ step in Scheme 2). In this particular case,

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however, one can show[46] from measured $k_D/k_N$ and $k_{-C}/k_N$[47] rate-constant ratios that the decomposition rate should be insensitive to the medium viscosity;[48] hence, the observed insensitivity to viscosity is consistent with a radical mechanism (for ONOOH). Using EPR spectroscopy, Augusto and coworkers have measured the formation of the $CO_3^{\bullet -}$ radical in near-quantitative yield from $ONOOCO_2^-$,[49,50] based upon limiting oxidation yields for its "indirect" reactions.[49] Earlier research in our[13] and Goldstein and Czapski's[51] laboratories has established that the reactive form of the adduct has chemical properties and reactivity characteristics consistent with those of the $CO_3^{\bullet -} + NO_2^{\bullet}$ radicals, and Goldstein and Czapski have also shown that product yields from "indirect" one-electron oxidations by $ONOOCO_2^-$ decreased markedly with increasing medium viscosity; this is consistent (in this case) with a radical mechanism.[52] Finally, Lehnig has observed chemically induced dynamic nuclear polarization (CIDNP) in the $^{15}N$ NMR spectrum of $NO_3^-$ during decomposition of $O^{15}NOOH$ and $O^{15}NOOCO_2^-$, which can be interpreted as indicating formation of $^{15}NO_3^-$ within the radical pairs $\{NO_2^{\bullet},OH^{\bullet}\}$ and $\{NO_2^{\bullet},CO_3^{\bullet -}\}$.[53] Thus, it seems appropriate to discuss the pressure dependencies of the reactions of ONOOH and $ONOOCO_2^-$ within the context of the radical pair mechanisms, i.e., Scheme 2. (Note that the rate-limiting step for the $ONOOCO_2^-$ reactions is adduct formation, whereas protonation is a pre-equilibrium step preceding rate-limiting peroxo O−O bond homolysis in the reactions of ONOOH.)

**Interpretation of the Pressure Dependencies of Product Yields.** According to Scheme 2, the product yields that are attained when all of the radicals escaping the cage are scavenged by reductants are dictated by the partitioning of the caged radical pair between cage escape ($k_D$) and rearrangement to $NO_3^-$ ($k_N$). The pressure dependence of the rate-constant ratios can be expressed as $\partial \ln(k_D/k_N)/\partial P = (\Delta V^{\ddagger}_N - \Delta V^{\ddagger}_D)/RT$ ($\equiv -\Delta(\Delta V^{\ddagger})/RT$, where $\Delta(\Delta V^{\ddagger})$ is the difference in volumes of activation for cage escape and rearrangement within the cage). From the pressure dependence of the maximal product yields ($Y_m$), given by the expression $Y_m = k_D/(k_N + k_D)$, one can calculate $\partial \ln(k_D/k_N)/\partial P$, hence $\Delta(\Delta V^{\ddagger})$. Results obtained for radical scavenging of ONOOH decomposition products by $Fe(CN)_6^{4-}$ and $Mo(CN)_8^{4-}$, and for $ONOOCO_2^-$ decomposition products by $Fe(CN)_6^{4-}$, are listed in Table 1. For ONOOH decomposition, $\Delta(\Delta V^{\ddagger})$ is independent of the identity of the scavenging ion and the reaction pH; this case is expected for the mechanism given in Scheme 2. In all cases, the values are large positive numbers, which indicates that greater volume expansion occurs during cage escape than during internal rearrangement. This result suggests that solvent restructuring constitutes a substantive energetic barrier to separation of the radical pair; although reasonable, this conclusion is not in accord with the frequently invoked assumption that, since the viscosity of water is nearly constant over this pressure range, $k_D$ will be pressure independent. This behavior markedly contrasts that of the direct bimolecular reaction between ONOOH and $Ni(cyclam)^{2+}$, the product yield for which is near stoichiometric and pressure independent, i.e., $\Delta(\Delta V^{\ddagger}) \simeq O$ (Table 1).

**Table 1.** Experimentally Determined Volumes of Activation

| reactant | pH[a] | $\Delta V^{\ddagger}_{obs}$ ($cm^3\ mol^{-1}$)[b] | $\Delta(\Delta V^{\ddagger})$[b,c] ($cm^3\ mol^{-1}$) |
|---|---|---|---|
| ONOOH | 4.5 | +6 | |
| ONOOH | 4.5 | +14[d] | |
| $ONOOH + Fe(CN)_6^{4-}$ | 4.5 | +12 | +13 |
| $ONOOH + Fe(CN)_6^{4-}$ | 7.2 | +10 | +14 |
| $ONOOH + Mo(CN)_8^{4-}$ | 7.2 | +11 | +16 |
| $ONOOH + Ni(cyclam)^{2+}$ | 7.2 | −7 | ∼0 |
| $ONOOCO_2^- + Fe(CN)_6^{4-}$ | 7.2 | −22 | +18 |

[a] In 0.15 M phosphate; other conditions for individual reactions as listed in the text. [b] Error limits are ±10−15%. [c] Defined as $\Delta V^{\ddagger}_D - \Delta V^{\ddagger}_N$ (Scheme 2). [d] Contained 5.0 mM added $NO_3^-$.

**Interpretation of the Pressure Dependencies of Reaction Rate Constants. (i) Bimolecular Reactions**. The experimentally measured bimolecular rate constant ($k_{obs}$) for the formation of $ONOOCO_2^-$ is given by $k_{obs} = k_2/\{(1 + [H^+]/K_a)(1 + K_b/[H^+])\}$,[8] where $k_2$ is the intrinsic rate constant for reaction between $ONOO^-$ and $CO_2$, $K_a = 2 \times 10^{-7}$ M is the acid dissociation constant for ONOOH, and $K_b = 1 \times 10^{-6}$ M is the constant for the $CO_2$ hydration−dehydration equilibrium, $CO_2 + H_2O \rightleftharpoons H^+ + HCO_3^-$. Under the experimental conditions (pH 7.2), this equation reduces to $k_{obs} \approx k_2[H^+]/K_b$. Since, in this case, the second acid dissociation constant ($K_a'$) for phosphate, $H_2PO_4^- \rightleftharpoons H^+ + HPO_4^{2-}$, is equal to $[H^+]$, it follows that $k_{obs} \approx k_2K_a'/K_b$, and $\Delta V^{\ddagger}_{obs} \approx \Delta V^{\ddagger}(k_2) + \Delta V^{\circ}(K_a') - \Delta V^{\circ}(K_b)$. The volumes of reaction for proton dissociation from $H_2PO_4^-$ and $CO_2$ hydrolysis are $\Delta V^{\circ}(K_a') = -24$ and $-27$ $cm^3\ mol^{-1}$, respectively.[54] Thus, the effects of pressure upon the two equilibria are mutually compensating and, from $\Delta V^{\ddagger}_{obs} = -22$ $cm^3\ mol^{-1}$, one calculates $\Delta V^{\ddagger}(k_2) \simeq -25$ $cm^3\ mol^{-1}$. This value is consistent with a reaction that involves association of a neutral molecule with an ion for which there is no significant change in charge density, hence, only minor contributions from solvent electrostriction. It therefore supports proposals that $CO_2$-catalyzed reactions of $ONOO^-$ involve intermediary formation of a discrete $ONOOCO_2^-$ adduct.[8,55]

The rate law for reaction of $Ni(cyclam)^{2+}$ with peroxynitrite is $d[Ni(cyclam)^{3+}]/dt = k_2'[ONOOH][Ni(cyclam)^{2+}]$;[16] the combination of a small $\Delta H^{\ddagger}$ with a large negative $\Delta S^{\ddagger}$ suggests that reaction occurs by an inner-sphere mechanism involving ONOOH coordination prior to electron transfer.[56] Following algebraic arguments analogous to those described above, for reaction of ONOOH with $Ni(cyclam)^{2+}$ at pH 7.2 we obtain the relationships $k_{obs} \approx k_2'K_a'/K_a$ and $\Delta V^{\ddagger}_{obs} \approx \Delta V^{\ddagger}(k_2') + \Delta V^{\circ}(K_a') - \Delta V^{\circ}(K_a)$. Using $\Delta V^{\ddagger}_{obs} = -7$ $cm^3\ mol^{-1}$ and $\Delta V^{\circ}(K_a) = -7$ $cm^3\ mol^{-1}$,[27] one obtains $\Delta V^{\ddagger}(k_2') \approx +10$ $cm^3\ mol^{-1}$. This large positive value may reflect loss of coordinated

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(46) Under the usual reaction conditions (see text), the $^{\bullet}NO_2$ and $^{\bullet}CO_3^-$ free radicals are effectively scavenged, so that $k_{-D} \approx 0$. Application of the steady-state approximation to the caged radical pair (Scheme 2) then gives $k_o = k_C(k_D + k_N)/(k_{-C} + k_D + k_N)$, where $k_o$ is the pH-independent first-order rate constant for ONOOH decomposition. In water, $k_N/k_D \simeq 1.5$ (determined from product yields for the "indirect" reactions) and $k_N/k_{-C} \simeq 1$,[47] so that $k_{obs} \simeq 0.6\ k_C$. Assuming that $k_D$ exhibits Debye−Stokes−Einstein behavior and the other microscopic rate constants are insensitive to viscosity, increasing the viscosity by 20-fold would increase $k_N/k_D$ to ∼30 for which $k_{obs} \simeq 0.5\ k_C$, i.e., causing the observed rate constant to decrease by less than 20%.

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(48) Additional factors which can reduce the magnitude of experimentally determined viscosity effects in this system (microheterogeneity, reactions of secondary radicals) are discussed in ref 52.

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(56) See, e.g.: Drljaca, A.; Hubbard, C. D.; van Eldik, R.; Asano, T.; Basilevsky, M. V.; le Noble, W. J. *Chem. Rev.* **1998**, *98*, 2167−2289.

water from the axial ligand positions of the complex ion accompanying ONOOH binding; typically, release of a single coordinated $H_2O$ is assumed to contribute $+13$ cm$^3$ mol$^{-1}$ to the overall $\Delta V^{\ddagger}(k_2')$.[57]

**(ii) Unimolecular Reactions.** The one-electron reductants, $Fe(CN)_6^{4-}$, $Mo(CN)_8^{4-}$, and $NO_2^-$, are efficient scavengers of the $OH^\bullet$ radical.[58,59] The mechanism given in Scheme 2 was kinetically modeled using reported rate constants[59] for reactions of $OH^\bullet$ and $NO_2^\bullet$. These calculations indicated that, even in the absence of other scavengers, $NO_2^-$ at levels above 1 $\mu$M will compete effectively with $NO_2^\bullet$ for $OH^\bullet$. Thus, $k_{-D}$ is negligible and the steady-state solution to Scheme 2 can be cast in the form $k_o = k_C(1 + k_D/k_N)/(1 + k_{-C}/k_N + k_D/k_N)$, where $k_o$ is the pH-independent first-order rate constant for ONOOH decomposition. At pH 4.5, $k_o \simeq k_{obs}$, and therefore $\Delta V^{\ddagger}_o \simeq \Delta V^{\ddagger}_{obs}$. Assuming that the pressure dependencies of both $k_{-C}$ and $k_N$ are small, the equality[47] $k_{-C} \approx k_N$ will hold at all pressures, and $\Delta V^{\ddagger}_o \approx \Delta V^{\ddagger}_C + RT\partial\ln[(1 + k_D/k_N)/(2 + k_D/k_N)]/\partial P$. Based upon the measured pressure dependence of product yields for the "indirect" reactions, the latter term is approximately $-1.5$ cm$^3$ mol$^{-1}$. The average of values obtained for $\Delta V^{\ddagger}_{obs}$ for reactions at pH 4.5 (Table 1) is $+11$ cm$^3$ mol$^{-1}$, which is nearly identical to the values in low phosphate environments reported by Goldstein et al.[27] Thus, the $H_2PO_4^-$ ion does not appear to influence the reaction pathway. One also notes that $\Delta V^{\ddagger}_{obs}$ is independent of whether $NO_2^-$ or $Fe(CN)_6^{4-}$ is the radical scavenger, as expected. From the expression for $\Delta V^{\ddagger}_o$, one obtains $\Delta V^{\ddagger}_C \approx +12$ cm$^3$ mol$^{-1}$. This value is somewhat larger than the estimated values of $\Delta V^{\ddagger}_C$ for single bond O−O cleavage in nonpolar environments, which are typically $+3-5$ cm$^3$ mol$^{-1}$,[60] but it is certainly consistent with rate-limiting formation of two neutral fragments via bond cleavage.

In weakly alkaline solutions, a competing pathway for ONOOH decomposition that gives rise to formation of $NO_2^-$ and $O_2$ becomes important. This pathway, which is thought to be initiated by reaction of $OH^\bullet$ with $ONOO^-$,[14,42] is completely suppressed upon addition of the $Fe(CN)_6^{4-}$ ion and other efficient radical scavengers.[42] Measured values of $\Delta V^{\ddagger}_{obs}$ in the presence of these scavengers are therefore expected to correspond to the steps leading to O−O bond homolysis, i.e., at pH 7.2, $k_{obs} \approx k_o K_a'/K_a$ and $\Delta V^{\ddagger}_{obs} \approx \Delta V^{\ddagger}_o + \Delta V^\circ(K_a') - \Delta V^\circ(K_a)$. Using $\Delta V^{\ddagger}_{obs} = +10$ cm$^3$ mol$^{-1}$ (Table 1) and the previously indicated $\Delta V^o$ values, one obtains $\Delta V^{\ddagger}_o \approx +27$ cm$^3$ mol$^{-1}$, independent of whether $Fe(CN)_6^{4-}$ or $Mo(CN)_6^{4-}$ is the radical scavenger. This value is substantially larger than both the $\Delta V^{\ddagger}_o$ determined at pH 4.5 in this study and the pH-independent values determined by Goldstein and co-workers in low phosphate environments.[27] These data therefore support earlier conclusions[26,27] that phosphate ions somehow modulate the reaction and suggest in particular that the interacting ion is $HPO_4^{2-}$. Ion-specific effects involving the $NO_2^-$ ion may also be important, since the difference in values obtained in media containing 0.05 and 5.0 mM $NO_2^-$, i.e., $+6$ and $+14$ cm$^3$ mol$^{-1}$, respectively (Table 1, Figure 1), appear to be outside the range of experimental uncertainty.

**Concluding Remarks.** The pressure dependencies measured for several "indirect" reactions of ONOOH are consistent with a mechanism in which peroxo O−O bond homolysis is the initial reaction step. These reactions are clearly distinguishable from $CO_2$-catalyzed oxidations by $ONOO^-$, whose pressure dependencies support conclusions based upon the rate law[8] that reaction is initiated by associative interaction of the oxidant and catalyst. Although most of the data can be accommodated by the scheme given in Figure 2, the observations that the product yields of the "indirect" reactions are pressure dependent, and that the $\Delta V^{\ddagger}_o$ values for several reactions are sensitive to the ionic composition of the medium, are difficult to rationalize within the context of this simple dynamical model and may indicate the existence of additional intermediates, e.g., solvent-separated radical pairs.

**Acknowledgment.** Funding for this research was provided by the National Institutes of Health under grant AI-15843 (to J.K.H.).

**Supporting Information Available:** A compilation of individual rate constants under various reaction conditions (Tables S1−S4), data plots of the pressure dependence of the $Fe(CN)_6^{3-}$ yield and rate constant for $Fe(CN)_6^{4-}$ oxidation by ONOOH (Figure S1), and the $Fe(CN)_6^{3-}$ yield from reaction between $ONOOCO_2^-$ and $Fe(CN)_6^{4-}$ (Figure S2), and the temperature dependence of the rate constant for reaction between ONOOH and $Ni(cyclam)^{2+}$ (Figure S3). This material is available free of charge via the Internet at http://pubs.acs.org.

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